

b.) Remarks

Claims 23-32 are presented in order to more specifically recite various preferred embodiments of the present invention. For the Examiner's reference, claims 23-25 correspond to item (e) of claim 1, claims 26-27, 28 and 29-32 respectively correspond to claims 6, 7 and 9, and the hybridization conditions recited in claim 27 are found at specification page 6, lines 1-7. Accordingly, no new matter has been added.

Claims 6-7 are objected to under 37 C.F.R. §1.75(c), as being improper dependent form for failing to further limit the subject matter of a previous claim. In response, these claims have been rewritten in conformity with the Examiner's suggestions. This, and the following rejection under Section 112 are the only issue presented.

Claim 6 is rejected under 35 U.S.C. §112, first paragraph, because the Examiner states those of ordinary skill are not capable of practicing the invention where "one to several amino acids are changed, added or deleted," or where the encoding DNA hybridizes under unspecified stringent conditions. These objections have all been addressed above: the reformulated claims which do not present "changed, added or deleted" language, and now specify the necessary hybridization conditions. <sup>1/</sup>

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition.

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<sup>1/</sup> The claim language is in conformity with U.S. Patent and Trademark Office practice, see U.S. Patent Nos. 5,399,346, 5,491,080, 5,631,236, 5,681,562, 5,693,536, 5,705,151, 5,811,273, 5,824,655, 5,827,702, 5,885,971, 5,922,685, 5,935,568, 5,985,846, 6,001,816, 6,017,896, 6,040,295, 6,048,524, 6,051,218, 6,054,288, 6,066,624, 6,080,728, 6,093,392, 6,093,699, 6,106,826, 6,150,130, 6,150,338, 6,159,467, 6,524,811, 6,593,304.

Simply as handy exemplars, see U.S. Patents Nos. 5,811,273 (claim 1), 6,150,130 (claim 1) and 6,524,811 (claim 1) which recite processes for producing a substance using a transformant harboring DNA encoding a protein providing a noted enzyme activity. Copies of these claims are attached at Tab A.


Accordingly, reconsideration and allowance of this application is earnestly solicited.

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Claims 23-32 remain presented for continued prosecution.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,

  
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USPTO PATENT FULL-TEXT AND IMAGE DATABASE

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United States Patent

5,811,273

Misawa , et al.

September 22, 1998

DNA strands useful for the synthesis of xanthophylls and the process for producing the xanthophylls

**Abstract**

Disclosed are the following DNA strands relating to the synthesis of keto group-containing xanthophylls such as astaxanthin and the like, and the techniques relating to the production of xanthophylls by genetic engineering: A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for converting a methylene group at the 4-position of .beta.-ionone ring into a keto group. A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for converting a methylene group at the 4-position of a 3-hydroxy-.beta.-ionone ring into a keto group. A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for adding a hydroxyl group to the 3-carbon of a 4-keto-.beta.-ionone ring. It is possible to produce a variety of xanthophylls such as canthaxanthin, astaxanthin and the like by introducing the DNA strands into an appropriate microorganism such as Escherichia coli and the like.

Inventors: Misawa; Norihiko (Yokohama, JP); Kondo; Keiji (Yokohama, JP); Kajiwara; Susumu (Yokohama, JP); Yokoyama; Akihiro (Shimizu, JP)

Assignee: Kirin Beer Kabushiki Kaisha (Tokyo-To, JP); Marine Biotechnology Institute Co., Ltd, (Tokyo-To, JP)

Appl. No.: 663310

Filed: September 23, 1996

PCT Filed: December 26, 1994

PCT NO: PCT/JP94/02220

371 Date: September 23, 1996

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PCT PUB.NO.: WO95/18220

PCT PUB. Date: July 6, 1995

**Foreign Application Priority Data**

Dec 27, 1993[JP]

5-348737

Sep 05, 1994[JP]

6-235917

United States Patent: 5,811,273

2/3 ページ

Intern'l Class:

C12P 007/26

Field of Search:

435/148,189,252.1,252.3,67,320.1,255.1,252.33  
536/23.1,23.2,23.7

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94/06918

Mar., 1994

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Misawa, et al. "Elucidation of the *Erwinia uredovora* Carotenoid Biosynthetic Pathway by Functional Analysis of *Gene* Products Expressed in *Escherichia coli*," *J. Bacteriology* 172(12): 6704-12 (Dec. 1990).

Misawa, et al. "Production of .beta.-Carotene in *Zymomonas mobilis* and *Agrobacterium tumefaciens* by Introduction of the Biosynthesis Genes from *Erwinia uredovora*," *Applied and Environmental Microbiology* 57(6): 1847-49 (Jun. 1991).

Misawa et al. "Structure and functional Analysis of a marine bacterial carotenoid biosynthesis *gene* cluster and astaxanthin biosynthetic pathway proposed at the *gene* level" *J. Bacteriol.* 177 (22), 6575-6584, Nov. 1995.

*Primary Examiner:* Wax; Robert A.

*Assistant Examiner:* Nashed; Nashaat T.

*Attorney, Agent or Firm:* Foley & Lardner

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Claims

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We claim:

1. A process for producing xanthophyll, comprising introducing a *DNA* strand into a microorganism having a canthaxanthin-synthesizing ability, wherein said *DNA* strand has a nucleotide sequence which encodes a polypeptide having an enzyme activity for adding a hydroxyl group to the 3-carbon of a 4-keto-.beta.-ionone ring, culturing the transformed microorganism in a culture medium, and obtaining astaxanthin or phoenicoxanthin from the cultured cells.
2. A process according to claim 1, wherein said microorganism is a bacterium or yeast.
3. A process according to claim 1, wherein said *DNA* strand has a sequence that codes for a polypeptide having the sequence of SEQ ID NO:4.
4. A process for producing xanthophyll, comprising providing a transformed microorganism wherein said transformed microorganism contains *DNA* that codes a polypeptide having an enzymatic activity for converting .beta.-carotene to canthaxanthin and that codes a polypeptide having an enzyme activity for adding a hydroxyl group to the 3-carbon of a 4-keto-.beta.-ionone ring, culturing the transformed microorganism in a culture medium, and obtaining astaxanthin or phoenicoxanthin from the cultured cells.

5. A process according to claim 4, wherein said microorganism is a bacterium.
6. A process according to claim 5, wherein said bacterium is selected from the group consisting of *Escherichia coli*, *Zymomonas mobilis* and *Agrobacterium tumefaciens*.
7. A process for producing xanthophyll, comprising introducing *DNA* that contains the *crtW* and *crtZ* genes isolated from *Agrobacterium aurantiacus* into a microorganism, culturing the transformed microorganism in a culture medium, and obtaining astaxanthin or phoenicoxanthin from the cultured cells.

## USPTO PATENT FULL-TEXT AND IMAGE DATABASE



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United States Patent  
Misawa , et al.

6,150,130  
November 21, 2000

DNA strands useful for the synthesis of xanthophylls and the process for producing the xanthophylls

### **Abstract**

Disclosed are the following DNA strands relating to the synthesis of keto group-containing xanthophylls such as astaxanthin and the like, and the techniques relating to the production of xanthophylls by genetic engineering: A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for converting a methylene group at the 4-position of a .beta.-ionone ring into a keto group. A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for converting a methylene group at the 4-position of a 3-hydroxy-.beta.-ionone ring into a keto group. A DNA strand having a nucleotide sequence which encodes a polypeptide having an enzyme activity for adding a hydroxyl group to the 3-carbon of a 4-keto-.beta.-ionone ring. It is possible to produce a variety of xanthophylls such as canthaxanthin, astaxanthin and the like by introducing the DNA strands into an appropriate microorganism such as Escherichia coli and the like.

Inventors: Misawa; Norihiko (Yokohama, JP); Kondo; Keiji (Yokohama, JP); Kajiware; Susumu (Yokohama, JP); Yokoyama; Akihiro (Shimizu, JP)

Assignee: Kirin Beer Kabushiki Kaisha (Tokyo-to, JP); Marine Biotechnology Institute Co., Ltd. (Tokyo-to, JP)

Appl. No.: 335919

Filed: June 18, 1999

### **Foreign Application Priority Data**

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5-348737

Sep 05, 1994[JP]

6-235917

Current U.S. Class:

435/67, 435/148; 435/189; 435/252.3; 435/252.33;  
435/320.1; 536/23.2; 536/23.7

Intern'l Class:

C12P 023/00; C12P 007/26

Field of Search:

435/189,148,252.3,252.33,67,320.1 536/23.2,23.7

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Misawa, N., et al., "Elucidation of the *Erwinia uredovora* Carotenoid Biosynthetic Pathway by Functional Analysis of *Gene* Products Expressed in *Escherichia coli*," *Journal of Bacteriology*, vol. 172, No. 12, pp. 6704-6712 (Dec. 1990).

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*Primary Examiner:* Nashed; Nashaat T.

*Attorney, Agent or Firm:* Foley & Lardner

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**Parent Case Text**

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This application is a divisional of application Ser. No. 09/006,491, filed Jan. 13, 1998, now U.S. Pat. No. 5,972,690 which is in turn a divisional of application Ser. No. 08/663,310, filed Sep. 23, 1996, now U.S. Pat. No. 5,811,273 which is in turn national stage of PCT/JP94/02220, filed Dec. 26, 1994.

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**Claims**

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What is claimed is:

1. A method for producing a xanthophyll comprising:

(a) introducing a *DNA* strand into a microorganism capable of synthesizing a .beta.-carotene, wherein the *DNA* strand encodes a polypeptide capable of converting the methylene group at the 4-position of a .beta.-ionone ring into a keto group,

(b) culturing the microorganism obtained in (a) in a culture medium, and

(c) obtaining canthaxanthin or echinenone from the microorganism cultured in (b).

2. The method of claim 1, wherein the polypeptide is found in *Agrobacterium* or *Alcaligenes*.

3. The method of claim 1, wherein the *DNA* strand hybridizes to the complement of another *DNA* strand comprising the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 8 in a solution comprising 5.times.SSC and 6.times.Denhardt for 16 hours at 60.degree. C. followed by washing in a solution comprising 2.times.SSC and 0.1% SDS for 1 hour at 60.degree. C.

4. The method of claim 1, wherein the polypeptide comprises the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 8.
5. The method of claim 1, wherein the .beta.-ionone ring is a 3-hydroxy-.beta.-ionone ring.
6. The method of claim 1, wherein the microorganism is a bacterium or a yeast.
7. A method for producing a xanthophyll comprising:
  - (a) introducing a *DNA* strand into a microorganism capable of synthesizing a zeaxanthin, wherein the *DNA* strand encodes a polypeptide capable of converting the methylene group at the 4-position of a .beta.-ionone ring into a keto group,
  - (b) culturing the microorganism obtained in (a) in a culture medium, and
  - (c) obtaining astaxanthin or 4-ketozeaxanthin from the microorganism cultured in (b).
8. The method of claim 7, wherein the polypeptide is found in *Agrobacterium* or *Alcaligenes*.
9. The method of claim 7, wherein the *DNA* strand hybridizes to the complement of another *DNA* strand comprising the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 8 in a solution comprising 5.times.SSC and 6.times.Denhardt for 16 hours at 60.degree. C. followed by washing in a solution comprising 2.times.SSC and 0.1% SDS for 1 hour at 60.degree. C.
10. The method of claim 7, wherein the polypeptide comprises the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 8.
11. The method of claim 7, wherein the .beta.-ionone ring is a 3-hydroxy-.beta.-ionone ring.
12. The method of claim 7, wherein the microorganism is a bacterium or a yeast.



**USPTO PATENT FULL-TEXT AND IMAGE DATABASE**

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United States Patent  
Cunningham, Jr. , et al.

6,524,811  
February 25, 2003

Methods of increasing or decreasing carotenoids and other isoprenoids using IPP isomerase

**Abstract**

The present invention describes the DNA sequence for eukaryotic genes encoding .epsilon. cyclase, isopentenyl pyrophosphate (IPP) isomerase and .beta.-carotene hydroxylase as well as vectors containing the same and host cells transformed with said vectors. The .epsilon. cyclase and .beta.-carotene hydroxylase genes disclosed include those from *A. thaliana*; the IPP isomerase genes disclosed include those from *A. thaliana*, *H. pluvialis*, and *marigold*. The present invention also provides methods for controlling the ratio of various carotenoids in a host cell and for the production of novel carotenoid pigments. The present invention also provides a method for screening for eukaryotic genes encoding carotenoid biosynthesis enzymes.

Inventors: Cunningham, Jr.; Francis X. (Chevy Chase, MD); Sun; Zairen (Hyattsville, MD)

Assignee: University of Maryland (College Park, MD)

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Filed: September 25, 1997

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Intern'l Class:

C12P 023/00

Field of Search:

536/23.2 435/233,320.1,325,67

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Sep., 1996

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Chem. Abstr. 125:294752, 1996.

*Primary Examiner:* Achutamurthy; Ponnathupura

*Assistant Examiner:* Kerr; Kathleen

*Attorney, Agent or Firm:* Arent Fox Kintner Plotkin & Kahn

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*Parent Case Text*

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This is a Division, of application Ser. No. 08/624,125 filed on Mar. 29, 1996, now U.S. Pat. No. 5,744,341.

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*Claims*

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What is claimed as new and is desired to be secured by Letters Patent of the United States is:

1. A method of obtaining a compound derived from dimethylallyl pyrophosphate (DMAPP), wherein said compound derived from DMAPP is an isoprenoid, steroid, or carotenoid, the method comprising:

(a) inserting into a host cell a vector comprising a heterologous nucleic acid sequence, which encodes a protein having isopentenyl pyrophosphate (IPP) isomerase activity, wherein the heterologous nucleic acid sequence is operably linked to a promoter;

(b) expressing the heterologous nucleic acid sequence to produce the protein wherein the protein enhances the production of a compound derived from DMAPP relative to an untransformed host cell;

(c) observing the host cell for a color change caused by the enhanced production of a compound derived from DMAPP; and

(d) recovering the compound derived from DMAPP from the host cell.

2. The method of claim 1, wherein the heterologous nucleic acid sequence has a sequence which encodes the amino acid sequence of SEQ ID NO: 14, 15, 16 or 18.

3. The method of claim 1, wherein the host cell is selected from the group consisting of a bacterial cell, an algal cell, a plant cell and a cyanobacterial cell.

4. The method of claim 1, wherein the host cell is a photosynthetic cell.

5. The method of claim 1, wherein the host cell is an E. coli cell.

6. A method of enhancing the production of a compound derived from DMAPP in a host cell, wherein said compound derived from DMAPP is an isoprenoid, steroid, or carotenoid, the method comprising:

(a) inserting into a host cell a vector comprising a heterologous nucleic acid sequence, which encodes a protein having isopentenyl pyrophosphate (IPP) isomerase activity, wherein the heterologous nucleic acid sequence is operably linked to a promoter;

(b) expressing the heterologous nucleic acid sequence to produce the protein wherein the protein enhances the production of a compound derived from DMAPP sufficiently to alter the visual appearance of the host cell by a color change relative to an untransformed host cell; and

(c) observing the host cells into which the vector has been inserted for said color change.

7. The method of claim 6, wherein the heterologous nucleic acid sequence has a sequence which encodes the amino acid sequence of SEQ ID NO: 14, 15, 16 or 18.

8. The method of claim 6, wherein the host cell is selected from the group consisting of a bacterial cell, an algal cell, a plant cell and a cyanobacterial cell.

9. The method of claim 6, wherein the host cell is a photosynthetic cell.

10. The method of claim 6, wherein the host cell is an E. coli cell.